APPENDIX B
WORK PLAN FOR ARSENIC BIOAVAILABILITY STUDY

Protocol for Bioavailability Study of Arsenic in CCBs Orally Dosed to Juvenile Swine

1 Project Identification Information and Approvals

Sponsor:
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2 Overview

This protocol describes an approach for determining the relative bioavailability of arsenic in soil that will be used to determine the relative bioavailability of arsenic in samples of coal combustion by-products (CCBs). Bioavailability refers to the amount of chemical that can cross a biological membrane (skin, gastrointestinal (GI) mucosa, alveolar walls) and enter the systemic circulation (*i.e.*, the absorbed dose). Bioavailability data are often a critical element of risk assessment and aid in the extrapolation between the results of toxicology studies involving one medium of exposure (*e.g.*, drinking water) and potential health effects associated with exposure to other media (*e.g.*, soil or dust). For metals in particular, bioavailability appears to be highly dependent on the matrix in which the metal is found. For example, it is well recognized that the bioavailability of metals in soil is reduced compared to the bioavailability of metals in aqueous solution (Steele *et al*., 1990; Freeman *et al*., 1995; Schilderman *et al*., 1997; Canady *et al*., 1997). The toxicity benchmarks for arsenic (*i.e.*, the reference dose and cancer slope factor) are based on human data from populations exposed to arsenic in drinking water, where the arsenic was present in a form with high bioavailability. In contrast, the bioavailability of arsenic from soil is expected to be comparatively low (see references above). Therefore, when risks from arsenic in soil or other non-aqueous matrices, such as CCBs, are of interest, obtaining information on matrix-specific bioavailability is important and can result in more appropriate estimates of potential health risks. This is done by incorporating the bioavailability estimate into the calculation of dose in the risk assessment.

Bioavailability may be expressed either in absolute terms (*i.e.*, absolute bioavailability) or in relative terms (*i.e.*, relative bioavailability). Absolute bioavailability is determined by comparing the amount of chemical present in the blood (or other tissue) after oral exposure to the amount present following intravenous exposure. Absolute bioavailability may also be determined by conducting a mass balance, by determining the mass of chemical contained within the whole body after dosing. This is rarely done, however, due to the large number of tissues which would have to be analyzed. Relative bioavailability is measured by comparing the amount of chemical present in the blood (or other tissue) after oral exposure to both the material of interest (*e.g.*, arsenic in soil) and to a highly soluble form of the chemical (*e.g.*, aqueous sodium arsenate). The ratio of the respective blood (or tissue) levels gives the relative bioavailability. Because chemicals in solid matrices are absorbed slowly and monitoring blood concentrations over long periods of time is difficult, determining absolute bioavailability for such chemicals is problematic. The bioavailability of chemicals in solid matrices is therefore normally assessed as relative bioavailability.

In most animals, including humans, absorbed arsenic is excreted primarily in the urine (ATSDR, 2000). The mass of arsenic excreted in the urine in both humans and animals appears to follow a linear pattern up to dose levels of at least 5,000 μg/day (USEPA, 1995). Thus, measurements of the urinary excretion fraction (UEF) in animals treated with arsenic in the matrix of interest and the reference material provide a means of estimating the relative bioavailability of arsenic. The relative bioavailability estimates can then be used in human health risk assessments to obtain a more appropriate estimate of potential human exposures.

3 Proposed Experimental Schedule

Study start (initiate dosing):
Day 1

In-life phase completion (animal termination):
2 weeks after the start of dosing

Sample analysis completion:
Approximately 5 weeks after animal termination (approximately 7 weeks after study start)

Final report:
Approximately 12 weeks after completion of sample analysis

4 Objectives

The objective of this study will be to use juvenile swine as a test system in order to determine the oral bioavailability of arsenic in CCBs relative to the bioavailability of soluble forms of arsenic. The relative bioavailability estimates will be used in estimating exposures to arsenic in CCBs for use in a human health risk assessment. The relative bioavailability of arsenic will be determined based on urinary arsenic excretion during 15 days of daily dosing. Relative bioavailability of arsenic in CCBs will be estimated by comparison to data from swine dosed with sodium arsenate for approximately 15 days under the same experimental schedule.

5 Experimental Design

This study involves subacute administration of a CCB-arsenic source in a 10-40 gram portion of diet as a means of characterizing the oral bioavailability of arsenic in CCBs relative to soluble arsenic. The oral route of administration was selected as the route of exposure because this is the most likely human route of exposure. A non-treated group will serve as a control for determining background arsenic levels. Five animals will be used in each treatment group. Treatment groups are summarized in Table 1. Two dose levels will be tested for sodium arsenate, and two dose levels will be tested for each CCB treatment group (there may be one or more types of CCBs tested using this protocol).

Table 1
Treatment Groups

Group (a)	Number of Animals	Dose Material Administered	Target Elemental Arsenic Dose (μg/kg-d)
1	5	Control	0
2	5	Sodium Arsenate	30
3	5	Sodium Arsenate	60
4	5	CCB Arsenic	60
5	5	CCB Arsenic	120

Note: Doses will be administered in two equal portions given beginning at 9:00 A.M. and 3:00 P.M. each day. Doses will be based on the mean weight of the animals in each group, and will be adjusted every three days to account for weight gain.
(a) If more than one type of CCB is evaluated using this protocol, two additional test groups will be added for each additional type of CCB.

The target arsenic doses identified in Table 1 were determined based on the Casteel *et al*. (1997b) study of arsenic in various soils and mining wastes. In that study, animal soil arsenic doses less than 50 μg/kg-d generally resulted in bioavailability estimates with large mean standard errors (*i.e.*, >10%). To obtain the target arsenic doses shown in Table 1, while using a reasonable amount of test material (*i.e.*, <10g), the concentration of arsenic in the test material should preferably be at least 90 parts per million (ppm) (Casteel, 2002a, personal communication), however, adjustments can be made to the dosing regimen to accommodate the testing of materials having lower arsenic concentrations.

6 Experimental Animals

6.1 Justification

The test system for this study will be male juvenile swine. Because the toxicology benchmark values for arsenic (*i.e.*, the reference dose and cancer slope factor) are derived from human data, an estimate of the likely human bioavailability of arsenic from CCBs is desirable. The swine model is preferable to other species (*e.g.*, the rat) because the GI tracts of the swine and human share many of the same physiologic features (Kararli, 1995; Weis and LaVelle, 1991; Dodds, 1982). For example, the pH values within the various segments of the GI tract are similar in pigs and humans (Kararli, 1995). In contrast, the pH of the rodent stomach and duodenum is higher than that of the human, presumably due the presence of a non-glandular forestomach and different patterns of gastric emptying (Kararli, 1995). Swine, including juvenile swine, are also a well established model for investigating GI tract diseases of concern to humans (Bertram *et al*., 1991; Darragh *et al*., 1995; Buddington *et al*., 1996; Helm *et al*., 2002). Furthermore, studies of the bioavailability of soil metals, including arsenic, have previously been conducted in swine (Casteel, *et al*. 1996, 1997a, 1997b, 2000, 2001, 2002b; Lorenzana *et al*., 1996) and the results of these studies have been used in USEPA risk assessments (USEPA, 1999). Finally, the weight and growth characteristics of juvenile swine are similar to those of young children (Miller and Ullrey, 1987), making them a highly appropriate model for this population. Thus, the swine model is an appropriate and accepted model for studying the bioavailability of arsenic in soil or other solid matrices such as CCBs.

6.2 Test System

Intact male swine of a genetically defined line, approximately 5-6 weeks of age at initiation of dosing, will be obtained from an appropriate vendor in sufficient numbers to provide the required number of healthy animals for testing (approximately 10 percent more than the number of animals to be tested). The target body weight at purchase will be 9-11 kg. The number of animals to be tested will be 25.

6.3 Animal Health and Quarantine

Animals will be held under quarantine to observe their health for one week before beginning exposure to test materials. Swine chosen for each investigation will be monitored throughout the investigation to identify any evidence of disease. The monitoring program will consist of the following elements:

- Daily observation by the Principal Investigator or designated veterinarian, with consultation as needed by a board-certified food-animal clinician. Observations for each animal will be recorded daily on a health-status chart attached to the cage of each animal. If any intervention is taken for an animal (*e.g.*, administration of antibiotics), this action shall be recorded on the chart for that animal.
- Any animal that dies during the study period will have a thorough postmortem examination conducted to determine the cause of death. The postmortem examination will include gross and histologic examinations, as well as any ancillary tests (such as microbiology) deemed appropriate by the veterinary pathologist. All observations and findings will be recorded.
- Veterinary records from the swine producer and the producer's veterinarian, including documentation of health status, will be available if needed to assess overall swine herd health, history of vaccinations, and other veterinary data.
- Pigs will be necropsied in the Veterinary Medical Diagnostic Laboratory (VMDL) by a board-certified veterinarian to determine cause of death. The VMDL is accredited by the American Association of Veterinary Laboratory Diagnosticians and is a full-service diagnostic lab with expertise in all aspects of animal disease.

6.4 Animal Housing

Animals will be individually housed in stainless steel, metabolic cages. Metabolic cages are designed to collect and separate urine and feces.

6.5 Diet and Water

Animals will be provided with 100 percent of their recommended daily food requirements. This will be achieved by supplying each animal with food equivalent to 4 percent of its body weight each day, in two equal portions beginning at 11:00 A.M. and 5:00 P.M. Because the animals are expected to grow significantly (0.3 to 0.8 kg/day) over the investigation period, the food portions must be adjusted upward over time. Two samples of each batch of feed delivered will be analyzed prior to usage to confirm low arsenic concentrations. Feed will be purchased from Zeigler Brothers, Inc., of Gardners, PA and detailed

analysis of the composition including concentrations of amino acids, vitamins and essential nutrients will be provided with each lot purchased.

In order to simplify feeding and to help maintain a constant body weight across different animals, administered food portions will be based on the mean body weight of all animals in the study. The mean body weight at three-day intervals will be used to calculate food intake for the following three days, adjusted by the expected weight gain between weighings, such that the mean body weight used is the estimated weight on day 2 of the 3-day period. Specifically, the twice-daily food portion will be calculated as follows:

On the day of the weighing,

$$\text{Portion } (\text{g}) = (\tfrac{1}{2})(0.04)(\text{body weight in kg})(1{,}000\ \tfrac{\text{g}}{\text{kg}})$$

This size portion will be used for the following three days, and then adjusted again in a similar manner.

Water will be provided to animals ad libitum *via* a pipe and nozzle that is activated by the animal. Laboratory technicians will check each day to ensure that all water delivery nozzles are functioning properly. The water source will be a municipal drinking water system. One water sample will be drawn at random from a drinking water nozzle once per week during the study and analyzed for arsenic.

6.6 Animal Identification

All of the animals will be uniquely identified by ear tag and cage tape. Each cage will be labeled with the number that corresponds with the ear marking of the animal in the cage.

6.7 Randomization

Animals will be randomly assigned to treatment groups by the following method:

- A list of animals will be prepared by ear tag number order.
- Random numbers will be generated by a computer and these numbers assigned to each animal's ear tag number.
- Animals will be sorted sequentially by assigned random number.
- The first five animals will be assigned to group 1, the next to group 2, *etc*.
- Animals will be sorted sequentially within assigned group by ear tag number.

7 Test Substances

7.1 Test Substance Identification

The test substance for this study will be samples of CCBs collected according to Sponsor requirements. These samples will be provided to the Test Facility by the Sponsor. Before the study, CCB arsenic will be characterized by the Test Facility as described in Section 7.2.

For the soluble arsenic-dosed study group animals, sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, molecular weight 311.91, CASRN 7631-89-2) will be used to administer appropriate doses of water-soluble forms of arsenic.

7.2 Test Substance Analysis

7.2.1 Test Sample Sieving

For bioavailability studies, only the fraction of the CCB sample in the <250-μm size range will be used to better represent material likely to adhere to children's hands and be ingested (Duggan and Inskip, 1985). Therefore, prior to test substance analysis and characterization, this fraction will be separated from the remainder of the sample by performing a standard sieve analysis using an ASTM No. 60 Sieve (60 openings per square inch of surface area). Material passing the No. 60 sieve corresponds to the fraction <250 μm.

7.2.2 Concentration

The concentration of arsenic in the test substances will be determined after completion of the sieving and physical mixing (see section 4.4.2). Analysis of the test substances will involve digesting (USEPA SWA—846—Soil Method) the sieved material, and measuring the concentrations of arsenic using either inductively coupled plasma atomic emission spectroscopy (ICP-AES) (USEPA SW-846 Method 6010B) or graphite furnace atomic absorption (GFAA) (USEPA SW-846 Method 7060A for arsenic [USEPA, 2000]). The concentrations of arsenic in the CCBs will be determined by taking 3 subsamples from different sections of the sieved CCB sample, digesting triplicate aliquots of each subsample, and analyzing single aliquots of the digestate. The concentration will be reported as the arithmetic mean of the three subsamples.

7.3 Dose Preparation

7.3.1 Soluble Arsenic Formulation

The appropriate amount of sodium arsenate stock solution will be mixed with a 5g (±1g) mass of moistened feed ("doughball"). The feed will be a special variety from Zeigler Brothers, Inc., of Gardners, PA. Mixture with the doughball will be achieved by placing the test material in a small depression in the doughball. After the stock solution has permeated into the doughball and no free liquid remains, the depression will be filled by squeezing the dough ball in on itself, and the doughball will be administered to the animal by hand feeding.

All animals in each dose group will receive the same volume of sodium arsenate stock solution, based on the mean body weight of all animals in the group. The precise dose to each animal will subsequently be calculated from the individual measured body weights. The total daily dose will be divided into two equal portions that will be administered in the morning and afternoon. The volume of the stock solution placed in the dough balls of each dose group will therefore be calculated using the following equation:

$$Vol = 0.5\left(\frac{MBW \times Dose}{Conc}\right)$$

where Vol = Volume of stock solution (μL)
MBW = Mean body weight (kg)
Dose = Target dose for the group (μg/kg-d)
Conc = Concentration of arsenic in stock solution (μg/μL)
0.5 = Division of the total daily dose into the two administered portions

Three stock solutions of sodium arsenate will be prepared at concentrations that will result in target dose concentrations of 25, 50, and 125 μg/kg when a volume of stock solution between 20 μL and 100 μL is added to the doughball.

7.3.2 CCB Formulation

The required mass of CCBs will be placed in a small depression in one or more 5-to-10-g (±1g) doughballs. Lower arsenic concentrations in CCBs may require additional doughballs to accommodate the appropriate mass of CCBs. The depression will be filled by squeezing the doughball in on itself, thereby trapping the test material in the center. If the mass of CCBs required is too large to encapsulate into a single doughball, the sample will be divided into approximately equal portions and placed in the minimum number of doughballs required to contain the CCBs.

All animals in each dose group will receive the same mass of test material, based on the mean body weight of all animals within the dose group. The precise dose to each animal will subsequently be calculated from the individual measured body weights. The dose will be divided into two equal portions that will be administered in the morning and afternoon. The mass of CCBs placed in the dough balls of each dose group will therefore be calculated using the following equation:

$$Mass = 0.5\left(\frac{MBW \times Dose}{Conc}\right)(1{,}000\ mg/g)$$

Where: Mass = Mass of CCBs (mg)
MBW = Mean body weight (kg)
Dose = Target dose for the group (μg/kg-d)
Conc = Concentration of arsenic in CCBs (μg/g)
0.5 = Division of the total daily dose into the two administered portions

7.4 Dose Analysis

7.4.1 Dose Verification and Stability

At least two extra doughballs (or sets of doughballs if more than one doughball is required to administer the material) will be prepared for each dose "batch" (a "batch" is a group of doughballs sufficient administration for three days). After all doughballs in the batch are prepared, two will be selected at random for dose verification, wrapped individually in plastic wrap, and placed together in a plastic bag labeled with the appropriate group/treatment identification number. All dose verification

samples will be stored in the freezer until the end of the study. At the end of the study, approximately 5 percent of verification samples will be randomly selected for analysis of arsenic.

7.4.2 Test Article Homogeneity

It is expected that the bulk CCB sample will be nonhomogeneous with respect to particle size, and the concentration and form of arsenic may vary as a function of particle size. Therefore, it is important that the CCB sample be well mixed prior to removal of the dose aliquots. This is achieved by placing the bottle containing the bulk CCB sample on a roller operating at low speed for about 20 to 30 minutes. After rolling, the bottle should be further mixed by inverting five times. It is important that vigorous methods of mixing not be used, because this might lead to alteration of the particle size distribution. Sample homogeneity will be assessed based on the concentrations reported in the three subsamples analyzed (see Section 7.2.2).

7.5 Dose Administration

Animals will be dosed twice daily for 15 days beginning at 9 A.M. and 3 P.M., with two minute intervals allowed for individual pig dosing, 2 hours before feeding. If uneaten portions of doughballs are discovered, these will be retrieved and offered again for consumption. The rare occurrence of refusal will be handled by retaining any leftover portion for arsenic determination.

8 Data Collection

8.1 Clinical Observations

Animals will be observed on a daily basis. Clinical observations of possible signs of toxicity will be recorded.

8.2 Food Consumption

Any fraction of food not eaten will be estimated and recorded.

8.3 Doughball Consumption

If a portion of the CCB-containing doughball is refused by the animals, that portion will be wrapped in plastic and retained for arsenic determination. The date, time and animal number will be recorded in the log book. The uneaten portion of the doughball will be homogenized by hand prior to analysis so that an estimate of the refused dose can be made.

8.4 Body Weights

Animals will be weighed every three days beginning at Day 0 of the study. Animals also will be weighed on the day of sacrifice. All body weights will be recorded in the laboratory log book to the nearest 0.1 kg.

8.5 Urine Collection

Urine (48-hour composites) will be collected from each animal on Days -2 to -1, 6 to 7, 8 to 9, and 10 to 11 of the study, beginning at either 9:00 or 10:00 A.M. on the first day of the collection period. Day 6 was selected as the start date for collection of urine from animals during treatment because previous studies conducted in swine to assess the bioavailability of arsenic have shown that urinary arsenic excretion patterns are stable after 5 days of dosing (Casteel *et al.*, 1997). Urine will be collected by placing a stainless steel pan beneath each cage that drains into a plastic storage bottle. Each collection pan will be fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris. During the 48 hour collection period, urine will be collected as needed (typically twice daily) from the collection pans and added to a collection container designated for each animal and stored in the refrigerator. At the end of the 48 hour collection period, the volume will be measured using a graduated cylinder and the urine volume recorded in the laboratory log book to the nearest 5 ml. The urine in the container will be mixed by swirling and three 60 ml aliquots will be removed and acidified with 0.6 ml concentrated nitric acid. One of the acidified aliquots will be archived in the refrigerator and one aliquot will be sent for arsenic analysis. The samples will be frozen at -20 ^{o}C until analyzed.

8.6 Tissue Collection

Following euthanasia on Day 15, animals will be necropsied with gross examination of the thoracic and abdominal organs. Fifty to 100 g of the right medial liver lobe and the entire right kidney will be removed and stored in plastic bags at -80 ºC. These tissues will be archived to allow for later reanalysis and verification, if necessary. Tissues will be collected in a manner that minimizes the potential for cross contamination (*e.g.*, the use of new or washed equipment to obtain tissues from each animal).

9 Analysis of Samples

9.1 Urine

All urine will be analyzed for total arsenic (urine) by atomic absorption on the hydride of arsenic (Brumbaugh and Walther, 1989). Twenty-five ml of urine will be transferred to an acid cleaned 100 ml beaker. Three ml of methanol will be added, followed by 5 drops of anti-foam agent, 10 ml of 40% (w/v) magnesium nitrate hexahydrate, and 10 ml of concentrated trace metal grade nitric acid (HNO_3). The sample will be covered with a watch glass and placed on a hot plate to reflux for 8-12 hours at 70 - 80 ºC or overnight. The temperature will then be increased to 200 ºC and the watch glass will be slid back to allow faster evaporation. The samples will be heated to complete dryness (8 - 12 hours), and then covered with the watch glass and allowed to cool. The samples will be transferred to a cool muffle furnace and ramped to 500 ºC at 1 degree/minute, then held at 500 ºC for 3 hours, turned off and allowed to cool. Samples will be removed and 5 ml D.I. water and 5 ml concentrated trace metal grade hydrochloric acid (HCl) will be added. Samples will be allowed to gently boil until the white residue is dissolved. After dissolving the residue, the samples will be cooled and diluted with de-ionized water to 50 ml. The samples will then be transferred to clean labeled 2 oz bottles.

When samples are ready for analysis they will be diluted for hydride generation AA with a solution of 10% HCl, 10% KI, and 5% ascorbic acid. The samples will initially be diluted l/10 or 1/5 in 10 ml, depending on the detection limit desired, and then capped. A 1/10 dilution should give a detection limit of 2 µg/L and a 1/5 should give a detection limit of 1 µg/L. Samples should set at least 30 minutes before analysis, but overnight is preferred. The hydride analysis will be carried out using a Perkin-Elmer 3100 AA with Fias 200 and AS90 accessories and according to the method supplied by the manufacturer.

Internal quality assurance samples will be run every tenth sample, and the instrument recalibrated every 15th sample. A blank, duplicate, and spiked sample will be run every 20th sample.

9.2 Other Tissues

If it is necessary to analyze liver and kidney for arsenic, the tissues will be analyzed as follows: Approximately 1 gram of kidney cortex or liver will be placed in a 100 ml beaker with 3 ml of methanol, 5 drops antifoaming agent, 10 ml of 40% (w/v) magnesium nitrate hexahydrate, 10 ml of trace metal grade concentrated HNO_3 and 2 ml of trace metal grade HCl. The beaker will be covered with a watch glass and heated on a hot plate at 70-80 ºC overnight. The watch glass will then be removed and the temperature increased to 200 ºC until the sample is completely dry (about 8-12 hours). The dried sample will be transferred to a muffle furnace and heated to 500 ºC at 1 degree/minute, then held at 500 ºC for 3 hours, turned off and allowed to cool. After cooling, 20 ml of 50% HCl will be added to restore the volume to 20 ml and this in turn will be diluted with water to 100 ml. Samples will be analyzed on the Perkin-Elmer 3100 as described previously.

10 Statistics

All individual raw data will be summarized and reported as the mean and standard deviation for each dose group.

The relative bioavailability (RBA) of arsenic will be calculated by measuring the amount of arsenic excreted in urine, the Urinary Excretion Fraction (UEF). The UEF is estimated by plotting mass recovered in urine per 48 hours divided by the amount given per 48 hours. The relative bioavailability equals the ratio of the slopes (UEFs) of the test material to the sodium arsenate:

$$RBA = UEF_{test} / UEF_{NaAs}$$

In this calculation, the slopes for the two compounds are used rather than point estimates in order to: (1) derive an RBA estimate valid across a dose range, and (2) account for variability in the data through the calculation of upper confidence limits. Use of the slope values requires an assumption of linearity, which as previously noted appears to be true for arsenic up to intakes of 5,000 μg/day.

In addition to calculation of the RBA, the 48 hour urinary arsenic elimination measurements may be compared between sodium arsenate and arsenic in the CCB matrix to confirm that the time to reach steady state elimination is not significantly different between the two matrices.

11 Sample and Records Retention

11.1 Sample Retention

All samples will be frozen (approximately –20 °C) and retained at the test laboratory or its designated facility until acceptance of the final report. At that time, all samples will be disposed of or shipped to the Sponsor as directed by the Sponsor.

11.2 Records Retention

All records that would be required to reconstruct the study and to demonstrate adherence to the protocol will be maintained at the test laboratory. The stipulations of this protocol will be implemented in with the spirit of USEPA's Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Standards (40 CFR 160). This study will be listed on the test laboratory's Master Study Schedule. All appropriate records will be maintained and will include, but not be limited to, the following:

- Quarantine and acclimation period information pertaining to daily housing and environmental conditions
- Animal body weights at randomization, animal identification, and source of animal supply
- Test substance inventory, receipt, and storage conditions [to include chain of custody for sample receipt from Sponsor]
- Original raw and reduced data on arsenic concentrations from all samples
- Dosing and sample collection times
- Food consumption, body weight, and clinical observation data
- Original raw and reduced data from test substance analysis upon receipt, dose preparation, and dose analysis
- A copy of the signed protocol
- All letters, memos, or notes that pertain to the study
- Original signed final report

Records will be maintained for a minimum period of 10 years. If the files are scheduled to be destroyed, ENSR Corporation will be contacted. If ENSR Corporation requests that the files be saved, the documents or copies of the documents will be sent to ENSR Corporation.

12 Reporting

A written final report of this study will be submitted to the Sponsor. The final report should include, but not be limited to:

- Objectives and procedures as stated in the approved protocol, including any amendments to the protocol.
- The source of supply, body weights, quarantine/acclimatization data, and comments regarding health and general behavior of the test animals.
- The number, sex, species and strain of animal used in the study.
- The levels, quantity and formulation of dosing preparations. A description of the dose administration and quantity per animal.
- The quantity of arsenic in dosing formulations and urine samples.
- Dates of quarantine, dosing, sample collection and sacrifice of the animals.

- A full description of the procedure used to produce data, including sensitivity, detection limits, and reproducibility.
- Food consumption, body weight and clinical observation data.
- Dated signatures of the Study Director and all senior personnel involved in the study.
- Dates on which the study began and ended.
- Name and address of the testing facility and the Sponsor.
- Location where the test was performed.
- Name of Study Director.
- Manufacturer, lot, and sample numbers of the sodium arsenate used for oral dosing; also, when appropriate, sample numbers for CCBs used for dosing.
- Summary of test CCB characterization data. Summary of statistical analyses of the data and conclusions drawn from the analyses.
- Deviations from the test facilities Standard Operating Procedures or of the approved protocol and their possible impact on the study.
- The location of the data archives.

13 Monitoring Visits

The Sponsor and/or the Sponsor's project manager may visit the performing laboratory periodically to monitor the progress of this study. The names of all persons representing the Sponsor and the dates of their visits will be documented by the test facility representatives.

14 References

Agency for Toxic Substances and Disease Registry (ATSDR). 2000. "Toxicological Profile For Arsenic." U.S. Department of Health and Human Services. Public Health Service Agency for Toxic Substances and Disease Registry, Atlanta, GA

Bertram, T.A., S. Krakowka, and D.R. Morgan. 1991. Gastritis associated with infection by Helicobacter pylori: comparative pathology in humans and swine. *Rev Infect Dis*. 13 Suppl 8:S714-22. Jul-Aug.

Brumbaugh, W.B. and M.J. Walther. 1989. Determination of arsenic and selenium in whole fish by continuous-flow hydride generation atomic absorption spectrophotometry. *J. Assoc. Anal. Chem.* 72(3):484-6.

Buddington, R.K., C. Malo, and H. Zhang. 1996. Prenatal and perinatal development of intestinal transport and brush border hydrolases in pigs. In *Advances in Swine in Biomedical Research* (Eds: M.E. Tumbleson and L.B. Schook), Plenum Press, New York.

Bull, D.C. 2000. The chemistry of chromated copper arsenate. I. Characterization of timber treatment plant chemical sludge. *Wood Science and Technology* 34:367-376.

Bull, D.C. 2001. The chemistry of chromated copper arsenate. II. Preservative-wood interactions. *Wood Science and Technology* 34:459-466.

Canady, R.A., J.E. Hanley, and A.S. Susten. 1997. ATSDR science panel on the bioavailability of mercury in soils: lessons learned. *Risk Anal.* 17(5):527-32.

Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E. Hoffman, W.J. Brattin, M.F. Starost, J.T. Payne, S.L. Stockham, S.V. Becker, and J.R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In *Advances in Swine in Biomedical Research* (Eds: M.E. Tumbleson and L.B. Schook), Plenum Press, New York.

Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E. Hoffman, W.J. Brattin, R.E. Guzman, M.F. Starost, J.T. Payne, S.L. Stockham, S.V. Becker, J.W. Drexler, and J.R. Turk. 1997a. Bioavailability of Lead to Juvenile Swine Dosed with Soil from the Smuggler Mountain NPL Site of Aspen, Colorado. *Fund. Appl. Toxicol.* 36:177-187.

Casteel, S.W., L.D. Brown, M.E. Dunsmore, C.P. Weis, G.M. Henningsen, E. Hoffman, W.J. Brattin, and T.L. Hammon. 1997b. Relative bioavailability of arsenic in mining wastes. Document Control No. 4500-88-AORH. U.S. Environmental Protection Agency, Region 8, Denver, CO.

Casteel, S.W., T. Evans, M.E. Dunsmore, C.P. Weis, B. Lavelle, W.J. Brattin, and T.L. Hammon. 2000. Relative bioavailability of arsenic in soils from the VBI70 site. Public Review Draft. U.S. Environmental Protection Agency, Region 8, Denver, CO.

Casteel, S., T. Evans, J. Turk, N. Basta, C. Weis, G. Henningsen, and E. Hoffman. 2001. Refining the risk assessment of metal-contaminated soils. *Int J Hyg Environ Health* 203(5-6):473-4.

Casteel, S.W. 2002a. Personal communication (Telephone conversation of Dr. Casteel, University of Missouri, with Dr. Schoof of Gradient Corporation.)

Casteel, W.W., T. Evans, S. Morris, S. Griffin, W. Brattin, and T. Hammon. 2002b. "Relative Bioavailability of Arsenic in Soils from Butte, Montana. Draft Report." USEPA, Region 8.

Cooper, P.A., D.L. Alexander, and T. Ung. 1993. What is chemical fixation? In *Chromium-Containing Waterborne Wood Preservatives: Fixation and Environmental Issues* (Eds: J. Winandy and M. Barnes), Forest Products Society, Madison, WI.

Darragh, A.J. and P.J. Moughan. 1995. The three-week-old piglet as a model animal for studying protein digestion in human infants. *J Pediatr Gastroenterol Nutr.* 21(4):387-93. November.

Davies, B.W. 1974. Loss-On-Ignition as an Estimate of Soil Organic Matter. *Soil Sci. Soc. Am. Proc.* 38:150.

Dodds, J.W. 1982. The pig model for biomedical research. *Fed. Proc*. 41:247-256.

Duggan, M.J., and M.J. Inskip. 1985. Childhood exposure to lead in surface dust and soil: a community health problem. *Public Health Rev.* 13:1–54.

Freeman, G.B., R.A. Schoof, M.V. Ruby, A.O. Davis, J.A. Dill, S.C. Liao, C.A. Lapin, and P.A. Bergstrom. 1995. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. *Fundam Appl Toxicol*. 28(2):215-22.

Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. In *Methods of Soil Analysis, Volume I: Physical and Mineralogical Analysis*, American Society of Agronomy, Inc., and Soil Science Society of America, Inc., Madison, WI. pp. 383-411.

Helm, R.M., G.T. Furuta, J.S. Stanley, J. Ye, G. Cockrell, C. Connaughton, P. Simpson, G.A. Bannon, and A.W.R. Burks. 2002. A neonatal swine model for peanut allergy. *J Allergy Clin Immunol.* 109(1):136-42.

Kararli, T.T. 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos*. 16(5):351-80.

Lebow, S.T., P.K. Lebow, and D.O. Foster. 2000. Part I: Leaching and environmental accumulation of preservative elements. In *Environmental Impact of Preservative-Treated Wood in a Wetland Boardwalk*, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. Research Paper FPL-RP-582. February. pp. 3-69.

Lorenzana, R.M., B. Duncan, M. Ketterer, J. Lowry, J. Simon, M. Dawson, and R. Poppenga. 1996. Bioavailability of arsenic and lead in environmental substrates. Office of Environmental Assessment, USEPA Region 10. EPA 910/R-96-002. February.

Miller, E.R. and D.E. Ullrey. 1987. The pig as a model for human nutrition. *Annu Rev Nutr*. 7:361-82

Schilderman, P.A., E.J. Moonen, P. Kempkers, and J.C. Kleinjans. 1997. Bioavailability of soil-adsorbed cadmium in orally exposed male rats. *Environ Health Perspect*. 105(2):234-8

Steele, M.J., B.D. Beck, B.L. Murphy, and H.S. Strauss. 1990. Assessing the contribution from lead in mining wastes to blood lead. *Regul Toxicol Pharmacol*. 11(2):158-90.

Stilwell, D.E. and K.D. Gorny. 1997. Contamination of soil with copper, chromium, and arsenic under decks built from pressure treated wood. *Bull. Environ. Contam. Toxicol.* 58:22-29.

United States Environmental Protection Agency (USEPA). 1995. "Bioavailability of Metals in Soils and Solid Waste. Standard Operating Procedure." Report prepared for the U.S. Environmental Protection Agency, Region VIII, by Roy F. Weston, Inc. Document Control No. 4800-045-018. June

United States Environmental Protection Agency (USEPA). 1999. "IEUBK Model Bioavailability Variable." Office of Emergency and Remedial Response. EPA 540-F-00-006. October.

United States Environmental Protection Agency (USEPA). 2000. "SW-846 On-Line: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods." Available at: http://www.epa.gov/epaoswer/hazwaste/test/main.htm. Last updated: August 2000.

United States Navy (USN). 2000a. "Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Navy and Marine Corps Facilities, Part 1- Final July 2000." Available on-line at:http://enviro.nfesc.navy.mil/erb/erb_a/support/wrk_grp/bio_a/bioa_guide_final1.pdf

United States Navy (USN). 2000b. "Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Navy and Marine Corps Facilities, Part 2- Final July 2000." Available on-line at: http://enviro.nfesc.navy.mil/erb/erb_a/support/wrk_grp/bio_a/bioa_guide_final2.pdf

Weis, C.P. and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In *The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters* 3:113-119